BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

APRIL 1938

THE PASTEUR-MEYERHOF REACTION IN MUSCLE METABOLISM

Harvey Lecture, November 18, 1937

EINAR LUNDSGAARD

Institute of Medical Physiology, University of Copenhagen

BEFORE ENTERING on my subject, may I say that this title is not quite adequate. The Pasteur-Meyerhof reaction is the main subject of my lecture, but it is my intention at the same time to deal with the metabolism of the aerobically working muscles on a somewhat broader basis than indicated by the title.

It is an assumption frequently encountered in physiological literature, that the metabolism of the aerobically working muscles is a pure carbohydrate metabolism. That assumption cannot have originated from the experience gained in metabolism determinations during muscular exercise. It is a familiar fact that even heavy muscular exercise can be performed on a pure fat diet, and with a respiratory quotient which indicates a combustion almost entirely of fat. Experiments by Krogh and Lindhard[1], and recently by their pupils[2,3], have demonstrated this very convincingly. Of course, the fact that hard muscular work can be performed under conditions in which the respiratory quotient is low, does not disprove the belief

that the working muscles oxidize carbohydrate only; for the possibility certainly exists that in the organism—presumably in the liver—there is a process of carbohydrate formation from fat, so that the muscles may very well be supplied with carbohydrate even in conditions in which the organism as a whole is metabolizing fat alone. However, this purely hypothetical possibility can scarcely explain why the conception of a pure carbohydrate metabolism in the aerobically working muscles is so hard to eradicate, as actually seems to be the case. From this remark perhaps you will anticipate that I think that this conception should be discarded.

If we endeavor to discover how such a conception arose, and whence it has obtained sustenance all this time, we shall quickly find that the conception of a pure carbohydrate metabolism in the striated muscles arises from, and is supported by, the experience gained in experiments on the chemistry of isolated muscles, mainly the isolated muscles of cold-blooded animals.

The first to make convincing demonstrations of the connection between the mechanical function of muscles and a production of lactic acid, i.e., a carbohydrate metabolism, were Fletcher and Hopkins[4]. At this juncture, however, perhaps I may be permitted to say that probably the first to express the supposition of a connection between a formation of lactic acid in the muscles and their work was the Swedish chemist Berzelius. Du Bois-Reymond[5] tells us that in 1841 Berzelius stated that he had noticed that muscles from animals which had been hunted before being killed, seemed to contain very considerable quantities of lactic acid, whereas muscles from partly paralyzed extremities seemed to contain only small quantities of that substance. Nevertheless, it was of course the famous investigations of Fletcher and Hopkins[4] that brought lactic acid formation into the prominent position in muscle chemistry which it has since preserved almost intact. Their experiments showed how lactic acid, which had accumulated in muscle during work, would disappear after the work when the muscle was kept under aerobic conditions. However, the explanation of the disappearance of the lactic acid under aerobic conditions stands to the credit of Meyerhof[6], from whose fine experiments it appeared, that under aerobic conditions lactic acid can be rebuilt into the glycogen from which it was originally formed. In forming an estimate of the value of these investigations, admirable as they are in themselves in their more general biological applicability, it must be remembered that the direct demonstration of the resynthesis of glycogen from lactic acid

can be made only under conditions in which the formation and the removal of the lactic acid are separated in time. Finally, it must be borne in mind that, judging from these experiments—on cold-blooded animals only, it is true—the resynthesis of lactic acid to glycogen seems to be a fairly slow process. It is now easy to see why Meyerhof's demonstration of a carbohydrate cycle: glycogen⇌lactic acid has become generalized, in the sense that such a cycle has been accepted as an explanation both of the so-called Pasteur reaction in yeast, and of the parallel circumstance that no lactic acid formation can be demonstrated in aerobically working muscles. If the theory should be maintained that a breakdown of glycogen to lactic acid is an absolutely essential phase in the chemistry of muscle contraction, it would become absolutely necessary to assume that a formation of lactic acid does take place also in aerobically working muscles; indeed a lactic acid formation of the same degree as that taking place in a muscle which, under anaerobic conditions, works with the same intensity. In actual fact, the entire conception of the central rôle of lactic acid in the chemistry of muscle contraction only became possible when Meyerhof's demonstration of the carbohydrate cycle permitted the assumption that the reason why no lactic acid formation can be demonstrated in an aerobically working muscle is, that the lactic acid actually formed is rebuilt into glycogen as quickly as it is formed. That the theory concerning the rôle of lactic acid formation in muscle contraction (for which Meyerhof fought, and especially in his controversy with Embden fought so energetically) necessitated this interpretation of the Pasteur-Meyerhof reaction in the muscles, and thereby made the same interpretation of the Pasteur reaction in yeast probable, is undoubtedly the psychological reason why this interpretation was accepted without hesitation. For actually, the direct proofs of the correctness of this view are not strong.

By the Pasteur reaction is understood, as you know, the circumstance that under aerobic conditions fermentation recedes, in some cases completely, so that the yeast has a metabolism that is purely oxidative. As far as we can see, Pasteur himself conceived fermentation and oxidation as two mutually independent metabolic processes, in the sense that they are not coupled processes, but two reactions which can replace each other. Nowhere does Pasteur express the supposition that the cause of the recession of fermentation under aerobic conditions is that the terminal products of the fermentation process, or intermediate products, are resynthesized into carbohydrate. That interpretation is Meyerhof's[6].

That the so-called oxidation coefficient, or the Meyerhof coefficient, is of fairly constant value, has been regarded as evidence that Meyerhof's interpretation of the mechanism of the Pasteur reaction is correct, that is, as evidence of a coupling between oxidation and resynthesis into carbohydrate. By the oxidation coefficient is understood the ratio between the recession of the fermentation or glycolysis under aerobic conditions and the oxidation intensity. The determination of this coefficient is of course of value only, when it concerns yeast or tissues in which a residue of the fermentation or glycolysis persists under aerobic conditions. It must be pointed out, however, that this coefficient displays nothing like pronounced constancy. And in those cases in which there has been an assumption that a conformity exists between the oxidation coefficient in yeast and tumor tissue, and the oxidation coefficient in muscle, it must be pointed out that no such conformity has been proved. The oxidation coefficient for muscle has been calculated on the basis of experiments on the directly demonstrable resynthesis of lactic acid to glycogen in muscle which, after having worked anaerobically, is supplied with oxygen. In the first place, the ratio found in such experiments between "disappeared lactic acid" and oxidation (oxygen consumed during restoration) is not at all constant; and in the second place, the question of how the basal metabolism of the muscle in the very long period of recovery is to be accounted for in the calculation is a most uncertain one. Finally, it must be pointed out, and that is perhaps the most important point, that the calculations are made on the supposition that the resynthesis of lactic acid is the only endothermic reaction in recovering muscles in which the oxidation energy is engaged, whereas we now know that other endothermic reactions take place (resynthesis of creatine phosphoric acid). All in all, it must be said that the postulated constancy of the oxidation coefficient in various tissues does not contain any demonstrable evidence that the Pasteur-Meyerhof reaction depends upon a coupling between a resynthesis of carbohydrates on the one hand, and oxidations on the other.

It has been asserted that the correctness of Meyerhof's interpretation of the mechanism of the Pasteur-Meyerhof reaction is proved by Meyerhof's experiments with yeast[7], the object of which was to show more directly an oxidative resynthesis to carbohydrate of the final product of the fermentation process, alcohol. Thus these experiments would correspond exactly with the demonstration of the oxidative resynthesis of lactic

acid to glycogen in muscles during aerobic recovery. Whereas in Meyerhof's muscle experiments[6] it has been possible to show directly an increase of the glycogen content in the muscles, proceeding in equal pace with the disappearance of the lactic acid, no corresponding direct demonstration has been made of an increase of the carbohydrate content in yeast respiring in an alcoholic medium. On the other hand, Meyerhof[7] under such conditions found a very low respiratory quotient, the respiratory quotient when yeast oxidizes alcohol usually being as low as 0.3. Meyerhof took this low quotient to be evidence of a resynthesis of carbohydrate, a reaction having the character of a partial oxidation and therefore bound to give a low respiratory quotient. From the respiratory quotient Meyerhof calculated the ratio between oxidized and resynthesized alcohol, that is to say the "oxidation coefficient", and found that it was of the same value as the oxidation coefficients in yeast calculated in other ways and the corresponding coefficients in tumor tissue and muscle.

I have made experiments of the same kind[8], and have been in a position to confirm the regular occurrence of very low respiratory quotients when yeast respires in alcoholic medium; at the same time, however, I consider I have been able to show that Meyerhof's interpretation of these very low quotients is incorrect.

If a moderate quantity of alcohol is employed in the medium, and the experiments are continued for some hours, it will be found that in the first periods the respiratory quotient is very low. At a certain point of time, dependent on the amount of alcohol added to the samples, the respiratory quotient rises quickly, rather suddenly and reaches unity. It can now be shown that as long as the respiratory quotient remains low, an organic acid accumulates in the samples, and, when the quotient rises, the organic acid begins to disappear while simultaneously the intensity of oxygen absorption begins to decrease. If the experiments are discontinued at a time when alcohol still remains in the samples, it is possible by direct alcohol determinations to establish the amount of alcohol that disappeared during the experimental period. From the carbon dioxide output and the directly determined acid formation (determined as increase in ether-soluble acids), it is possible to calculate the total amount of alcohol oxidized completely and that oxidized to organic acid. If the sum of these two values is compared with the directly determined loss of alcohol, we find that the latter is somewhat greater than the sum of the other two values. Thus some alcohol seems to have disappeared which cannot be

accounted for, and which consequently may have been converted into carbohydrate. This quantity, however, is much smaller in proportion to the alcohol oxidation than that calculated by Meyerhof. The latter calculated the ratio between alcohol resynthesized into carbohydrate and alcohol oxidized at 3 : 1; according to my experiments the ratio cannot be more than 1 : 1, that is to say a ratio of dimensions quite different from the oxidation coefficient found previously. There can be no doubt that the very low respiratory quotients are mainly a result of the fact that alcohol is oxidized by yeast in two phases, the alcohol first being converted into organic acids (presumably acetic acid), which thereupon are oxidized completely.

TABLE I

	CO_2 cmm	O_2 cmm	RQ	*Alcohol completely oxidized* mg%	*Alcohol oxidized to acid* mg%	*Alcohol disappeared calculated* mg%	*Alcohol disappeared determined* mg%	*Alcohol oxidized to carbohydrate* mg%
POISONED SAMPLES								
Exp. 1	93	280	0,33	9,5	21,1	30,6	31	0
" 2	72	289	0,25	7,4	29,0	36,4	34	—2,4
" 3	85	288	0,30	8,7	27,6	36,3	33	—3,3
" 4	116	348	0,33	11,9	23,6	34,5	38	3,5
" 5	117	338	0,35	12,0	24,4	36,4	33	—3,4
NORMAL SAMPLES								
Exp. 1	167	499	0,34	17,1	22,1	39,2	56	16,8
" 2	178	500	0,36	17,8	23,4	41,2	58	16,9
" 3	173	495	0,35	17,7	25,8	43,5	56	12,5
" 4	130	349	0,38	13,3	14,7	28,0	38	10,0
" 5	138	372	0,37	14,1	17,0	31,1	35?	3,9?

As I attach some importance to these experiments I shall take the liberty of going into them a little further and showing you some of the numerical data. The experiments were carried out partly with normal yeast and partly with yeast poisoned with iodo-acetic acid, whereby the ability of the yeast to ferment is destroyed. In table 1 you see the results of five experiments with normal yeast and five with poisoned yeast. The last column contains the calculated formation of carbohydrate. You will observe that in the poisoned samples, which by the way have a rather lower oxygen absorption than the normal samples, there is no evidence of any carbohydrate formation, whereas in the normal samples there is. The ratio of alcohol oxidized to alcohol resynthesized appears from a comparison between the fourth and the last column. There is no difference, how-

ever, in the respiratory quotient in the two series of experiments. Thus we may say that the respiratory quotient in the normal samples does not appear to reflect any carbohydrate synthesis. Only when we make the rather complicated calculation, the elements of which are the output of carbon dioxide, the alcohol determinations and those of the ether-soluble acids, is any carbohydrate formation revealed. In advance it was reasonable to assume that there would be no carbohydrate formation, at any rate in yeast poisoned with iodo-acetic acid, and no such formation could in fact be demonstrated. Therefore, the consequence of the close agreement between the value of the respiratory quotient and its variations in experiments with normal and poisoned yeast is, that we cannot feel fully convinced that carbohydrates really are formed in the normal samples.

TABLE II

	Poisoned			Normal		
	cmm CO_2	*cmm* O_2	*RQ.*	*cmm* CO_2	*cmm* O_2	*RQ.*
0 min. to 30 min.	37	114	0,32	39	112	0,35
35 min. to 1 hr. 05 min.	25	110	0,23	44	132	0,33
1 hr. 10 min. to 1 hr. 40 min.	29	101	0,29	61	150	0,41
1 " 45 " " 2 " 15 "	43	80	0,54	84	86	0,98
2 " 20 " " 2 " 50 "	47	55	0,86	76	76	1,00
2 " 55 " " 3 " 25 "	40	43	0,93	30	29	1,03
3 " 30 " " 4 " 00 "	40	41	0,98	21	21	1,00
4 " 00 " " 4 " 30 "	31	33	0,94	9	9	1,00
4 " 30 " " 5 " 00 "	28	27	1,03	8	8	1,00
5 " 00 " " 5 " 30 "	16	15	1,05			
5 " 30 " " 6 " 00 "	8	8	1,00			

In table 2 I have shown the results of two experiments with normal and poisoned yeast, continued so long that the alcohol disappeared entirely; the oxidation intensity has fallen to the same low values as are found for yeast suspended in pure phosphate buffer. In other words, the experiments were continued till the samples were practically free of substrate. You will observe that the quotient rises through the experiment—rather more gradually in the poisoned sample than in the normal one. If we calculate the quotient for the total experimental period we get 0.54 for the poisoned sample and 0.59 for the normal one, that is to say, quotients that are rather below the quotient for a pure alcohol oxidation, but of almost equal size in the two samples. Were the reduction of the quotient due to a formation of carbohydrate, the latter would, according to these experiments, in which the calculation is not very complicated, be just as pronounced in

the poisoned sample as in the normal. There is nothing to show that the organic acid in the normal sample passes through a conversion to carbohydrate before being completely oxidized. I therefore consider that my experiments entitle me to conclude that, even if one cannot definitively deny the possibility of a synthesis of alcohol to carbohydrate, no real evidence can be produced to show that such a process does occur. If a synthesis of alcohol to carbohydrate does occur, at any rate the oxidation quotient of that process has a value quite different from the one Meyerhof[7] calculated, and from the one which can be calculated on the oxidation intensity and the recession of the fermentation when yeast respires in carbohydrated medium. Consequently, nothing in these experiments tends to show that Meyerhof's interpretation of the mechanism of the Pasteur-Meyerhof reaction is correct, and therefore there is some justification in asking if there is any reason for continuing to accept that interpretation.

You will recollect my saying that Meyerhof's interpretation was, so to speak, necessary if the conception of lactic acid formation as an essential part of the chemistry of muscle contraction were to be maintained. Here again it is not possible today to discern any reason for persisting in this interpretation.

At the present moment it is doubtless generally acknowledged that the formation of lactic acid is not essential to the chemistry of muscle contraction. I believe there is reason for saying that the introduction of iodo-acetic acid poisoning in muscle chemistry has contributed very considerably to the rapid and complete acceptance of that view.

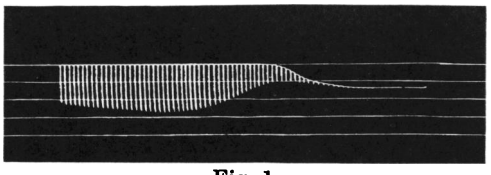

Fig. 1

Typical series of isometric twitches performed by a gastrocnemius muscle poisoned with iodo-acetic acid. Muscle weight: 0.7 g. Distance between two horizontal lines: 200 g. tension.

Though for obvious reasons the investigations on the chemical changes in working muscle poisoned with iodo-acetic acid holds a favorite place in my heart, on this occasion I shall deal only briefly with the results achieved from the experiments[9,10].

The effect of iodo-acetic acid is an inactivation of the enzyme system involved in the formation of lactic acid. A muscle poisoned with this compound is capable of performing a series of 60-90 twitches anaerobically without any formation of lactic acid taking place and then goes into rigor (Figure 1). The twitches are in all respects completely normal, that is in respect of latent time, duration of the twitch, heat formation, development of tension and action current. The total amount of work which an isolated poisoned muscle is able to perform is, however, smaller than that which a normal one can perform. This may be expressed by saying that the energy reserve available under anaerobic conditions is decreased when the ability to form lactic acid is abolished. These facts alone give a clear conception of the rôle played by the formation of lactic acid in the chemistry of muscle contraction. The formation of lactic acid is not a necessary link in that chemistry, and consequently this process cannot have any direct connection with what we may call the mechanism of contraction. On the other hand, the formation of lactic acid or the ability to form lactic acid must be looked upon as an important energy reserve available under anaerobic conditions.

When we determine the chemical changes occurring in an iodo-acetic poisoned muscle during stimulation, we find that the intensity of the breakdown of creatine phosphoric acid is greater than in a normal muscle. When a poisoned muscle has performed a series of 60-90 twitches the muscle is exhausted and goes into rigor. At this stage the total amount of creatine-phosphoric acid has been broken down, and, moreover, the adenylic phosphoric acid has been broken down too. In a normal muscle only about one-third of the creatine phosphoric acid has been broken down after the performance of 60-90 twitches. The energy liberated during the anaerobic work of a poisoned muscle undoubtedly originates from the breakdown of creatine phosphoric acid. Having regard to the time I have at my disposal I shall not venture upon a discussion of this statement.

There is one point, however, elucidated through my experiments with muscle poisoned with iodo-acetic acid, which I would like to deal with more fully, as it is of essential importance to the subject of my lecture today.

When two symmetrical sartorii, which have been poisoned by soaking in Ringer solution to which iodo-acetic acid has been added, are stimulated, one in oxygen and the other in nitrogen, it is seen that the muscle

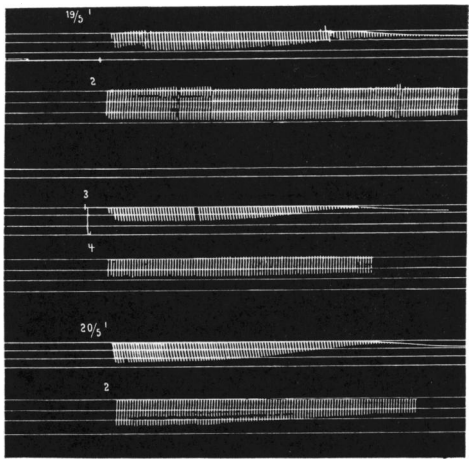

Fig. 2

Series of isometric twitches performed by symmetrical IAA-poisoned sartorii muscles, one stimulated in nitrogen (upper ones) and one stimulated in oxygen (lower ones).

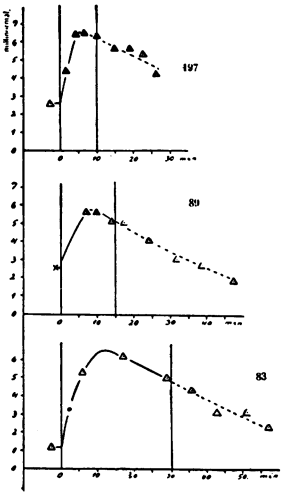

Fig. 3

Changes in blood lactate concentration during and after muscular exercise. Duration of work indicated by interval between the two vertical lines. Working intensity in all experiments 900 kgm/min.

supplied with oxygen is capable of performing much more work than the muscle stimulated in nitrogen. The latter will be completely exhausted and go into rigor at a time when the other muscle is still giving maximum tension. This is illustrated by these records, from experiments of the kind I have described (Figure 2). If the muscle which has worked aerobically is analyzed at a point of time when it has performed a much greater work than the completely exhausted anaerobic muscle, it will be found still to contain considerable quantities of creatine phosphoric acid. This means that there can be no doubt that energy arising from oxidation processes can be utilized in the production of mechanical energy, that is to say, utilized for the performance of exterior work, even under circumstances where there can be no question of intermediate lactic acid formation. That such a formation is out of the question, may be concluded with certainty from the fact that the symmetric muscle, working under anaerobic conditions, passes through the series of twitches in a manner characteristic of a muscle completely poisoned with iodo-acetic acid.

We cannot say with certainty whether under these circumstances the oxidation energy is utilized through an oxidative resynthesis of creatine phosphoric acid, or is transferred more directly to what we may call

"the mechanism of contraction". As long as it has not been shown that the breakdown of creatine phosphoric acid is not a necessary link in the chemistry of muscular contraction, I shall consider it most natural to assume that the oxidation energy is utilized through an oxidative resynthesis of creatine phosphoric acid; but perhaps the day is not far distant when it will be possible to show that the breakdown of creatine phosphoric acid is not an obligatory process in the chemistry of muscle contraction, just as it has been possible to show this as far as lactic acid formation is concerned.

To my mind the following conclusions are justified from the results obtained in experiments with muscles poisoned with iodo-acetic acid.

The formation of lactic acid is not an essential element in the chemistry of muscle contraction, nor is an intermediate lactic acid formation a necessary element in the utilization of oxidation energy for the production of mechanical energy in the muscles.

Accordingly, the experience gained in experiments with isolated muscle no longer provides a reason for assuming that in aerobically working muscle there is a constant intermediate formation of lactic acid which, as quickly as it is formed, is resynthesized to glycogen. Another way of expressing this is, that no reasons can be found for accepting Meyerhof's interpretation of the Pasteur-Meyerhof reaction in aerobically working muscles. On the other hand we can still speak of a Pasteur-Meyerhof reaction in aerobically working muscles, as by this we merely understand the circumstance that the formation of lactic acid decreases in the muscles under aerobic conditions.

The question may now be asked: If we cannot accept the factors which originally supported the "classic" view of the Pasteur-Meyerhof reaction in the striated muscles, then are there any others to be advanced in favor of that view? I do not think so.

There are experiments on the blood lactic acid concentration in man during and after muscular exercise, and these experiments to my mind rather argue against the classic view of aerobic muscle metabolism. Experiments of this kind were made by O. Bang[11], partly in Krogh's institute and partly in mine.

By means of a micro-method, which makes it possible to measure the lactate concentration in blood samples of only 0.1 cc., Bang has followed the variations in the blood lactate concentration during and after exercise. Thanks to the method employed, the concentration could be deter-

mined at short intervals, whereby a reliable curve was obtained for the variations. As far as the present subject is concerned, greatest interest attaches to those experiments in which the working intensity was such that a "steady state" was quickly arrived at. Figure 3 shows three curves from such experiments. It will be seen that immediately after the commencement of the exercise the blood lactate rises quickly and reaches a maximum in the course of from seven to ten minutes. Then the concentration begins to fall, even when the work is continued. This means, then, that even under conditions in which a steady state is arrived at for oxygen absorption, this does not happen with the lactic acid concentration, at any rate, not in the blood. In experiments in which the working intensity is less, and the primary increase of the blood lactate is consequently lower, the blood lactate falls even to basal values during the work. Even if we cannot assume that the blood lactate at any moment reflects the concentration in the working muscles, the falling blood curves to me are incompatible with the conception of a high, constant level in the working muscles throughout the working period. This applies especially to the experiments in which the blood lactate fell to basal values during the work. Now, this high and constant level is necessary if the classic view of the metabolism of the aerobically working muscles is to be upheld. According to that view, the assumed constant lactic acid production was supposed to lead in relatively few minutes to the attainment of a constant, increased lactate level in the active muscles, subsequently maintained throughout the "steady state". The constant level, it was thought, was attained by the rise in lactate concentration in itself inducing a rise in the intensity of oxidation, which in turn would involve a rise in the lactate resynthesis, until an equilibrium was established between production and resynthesis, at a level depending on the intensity of lactic acid formation, i.e., the intensity of work.

It is an outstanding feature of the curves in Figure 3 that the fall in blood lactate continues steadily after cessation of the work. This is difficult to reconcile with the conception that during work a very intensive production of lactic acid proceeds in the active muscles, but that this ceases simultaneously with the work. In that case one would expect to find a much quicker fall after work ceased. In reality, however, the three curves are almost identical, although they represent experiments with varying periods of work. The working intensity, on the other hand, was the same in all three experiments.

Personally, I am inclined to believe that Bang's interpretation of the experiments may be correct. According to Bang[11], we must assume that an intensive formation of lactic acid takes place in the active muscles in the first minutes after the commencement of work. Presumably the reason is that it takes a certain time for the general and local circulations to adapt themselves to the increased demand for oxygen. Until the circulation is adjusted, the muscles work partly anaerobically; that is to say they utilize the anaerobically available energy depôt represented by the ability to form lactic acid. When the circulation is adjusted and the oxygen supply to the muscles is adequate, all formation of lactic acid ceases. Thus, at the commencement of work a depôt of lactic acid is formed in the active muscles. The size of this depôt is independent of the duration of the work, and solely dependent on the intensity of the work. The greater the intensity of the work, the greater quantity of lactic acid can be formed before the circulation has become adjusted.

However, even if there is much in favor of Bang's interpretation of the experiments, it must be said that not even that interpretation provides a fully satisfactory explanation of the circumstance that the fall of blood lactate continues steadily after cessation of work. The depôt of lactic acid which, according to Bang, is presumably formed in the active muscles during the first minutes of the work, must be imagined as being removed, partly by diffusion to the blood and from there to resting organs (principally the liver), partly by oxidation in the active muscles, and finally by resynthesis to glycogen in the active muscles. Whereas the latter process may well be a slow one, it is hard to understand that the oxidation of lactic acid in the active muscles does not proceed with considerable intensity, and especially with greater intensity during work, when metabolism is greatly increased, than after cessation of work. A dependence of the rate of lactic acid removal on the rate of metabolism actually has been demonstrated by Newman, Dill, Edwards and Webster[12].

For this reason the fact that the blood lactate concentration may fall and even reach the basal level during the working period must be considered the most clearcut and consequently the most important result of the determinations of the blood lactate concentration during muscular exercise. As already mentioned this observation is incompatible with the classic view, according to which the metabolism of aerobically working muscles includes an obligatory intermediate lactic acid formation, which is balanced by an oxidative resynthesis of the lactic acid formed.

If now we return to the theory of a pure carbohydrate metabolism in the active muscles, it must be said that we have been unable to find any support for that theory in the experimental facts to which I have so far referred. This is a matter of considerable weight, as it eliminates the basis on which the theory to my mind has principally rested.

As I have laid particular stress on Meyerhof's interpretation of the Pasteur-Meyerhof reaction as a basis for the theory of a pure carbohydrate oxidation in aerobically working muscles, I should state at the same time that this interpretation, as indeed pointed out by Meyerhof too, does not require as an inevitable consequence that the metabolism of the active muscles be a pure carbohydrate oxidation. There is the possibility that the energy necessary for the endothermic lactic acid synthesis is produced by the oxidation, not of lactic acid or carbohydrate, but of fat or protein. This, however, does not alter the fact that Meyerhof's interpretation of the Pasteur-Meyerhof reaction has been regarded, and justly so I think, as an argument in favor of the assumption that the aerobically working muscles oxidize carbohydrate only.

Personally, I am inclined to the opinion that under such conditions, in which a formation of lactic acid actually takes place in the active muscles, the effect is that the oxidation proceeds entirely, or principally, at the expense of carbohydrate. In muscle working partly anaerobically there will be simultaneously lactic acid formation and oxidation. With very intense muscular exercise, which can only continue for a short time, the muscles will work to an appreciable degree anaerobically, and under just such conditions we see respiratory quotients of about unity, even when the quotient in rest lies much lower. Exactly the same thing is seen in the untrained subject during moderate work. Here, too, the respiratory quotient rises and remains high during the work, and simultaneously the blood lactate curve rises steadily as a sign that the muscles are working under partly anaerobic conditions.

If it be true, as I am inclined to believe, that lactic acid formation in the active muscles always leads to a high respiratory quotient, that is to say, to a combustion of carbohydrate; then it must be said that the low respiratory quotients seen in well-trained individuals on a fat diet, even during the performance of heavy work, also indicate that lactic acid formation is no obligatory link in the metabolism of the active muscles.

In conclusion, I would refer to a matter which, to me, has been of no small importance in judging the question of whether there is reason for

assuming that the metabolism of the aerobically working muscles is of specific character. For if it is, the most natural conclusion to draw is that it is a pure carbohydrate metabolism.

What I have in mind in this connection is the fact that the intensity of alcohol oxidation is not affected by muscular exercise.

I admit that the question of whether muscular exercise affects the intensity of alcohol oxidation has been answered variously by different authors. Nevertheless, in recent years more and more have accepted the view that the question must be answered in the negative. Among the experimental results which seem to show that the intensity of alcohol oxidation is not increased during muscular exercise, those of Nyman and Palmlöv[13] are particularly convincing. As shown in experiments by Widmark[14] and others, however, the intensity of alcohol oxidation increases if metabolism is stimulated by dinitrophenol. This is also the case with thyroxin, according to Le Breton's preliminary experiments[15]. As alcohol oxidation represents up to 80 per cent of the basal metabolism, it would seem necessary to assume that part of the alcohol oxidation proceeds in the muscles. Therefore, the circumstance that an increased metabolism conditioned by muscular exercise does not increase the alcohol oxidation, whilst an increased metabolism produced by stimulating drugs does, seems to justify the conclusion that there is a specific difference between the metabolism of the resting muscles and that of the active muscles. In her monograph on alcohol oxidation Le Breton[15] actually arrives at a conclusion to this effect.

The circumstance that muscular exercise does not increase the intensity of alcohol oxidation might, however, have the explanation that neither in rest nor in work is alcohol metabolized directly in the muscles. If we imagine that alcohol oxidation is a specific function of the liver, it is easy to understand that an increased metabolism, due to an increased energy output in the muscles alone, does not increase the alcohol oxidation, whereas an increase of the metabolism intensity in all tissues, the liver included, does involve an increase of the alcohol oxidation.

One difficulty in assuming that oxidation of alcohol takes place only in the liver, is that the absolute quantity of alcohol oxidized in rest, as already stated, represents up to 80 per cent of the basal oxygen absorption, whereas in the liver the oxygen absorption amounts to only 30 or 40 per cent of the total basal oxygen absorption. Therefore, the assumption that alcohol oxidation is a specific liver function is conditional upon the

alcohol oxidation in the liver being a partial oxidation. A priori that idea cannot be dismissed, so much the more as partial oxidations are undoubtedly a characteristic feature in the metabolism of the liver. Even if we must also assume that the partially oxidized product formed in the liver is oxidized in all tissues, the muscles included; the primary oxidation in the liver will nevertheless be the limiting factor for the disappearance of alcohol in the whole system.

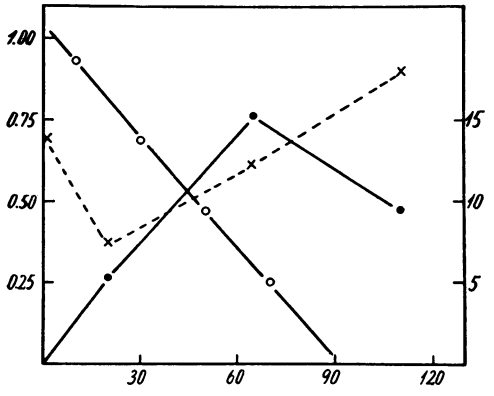

Fig. 4

Exp. on alcohol oxidation in isolated cat liver. Abscissas: time in minutes. Left ordinate: blood alcohol in per thousand and RQ. Right ordinate: decrease in alkali reserve in cc carbon dioxide.

o —— o blood alcohol

x ---- x R.Q.

● —— ● acid formation (decrease in alkali reserve)

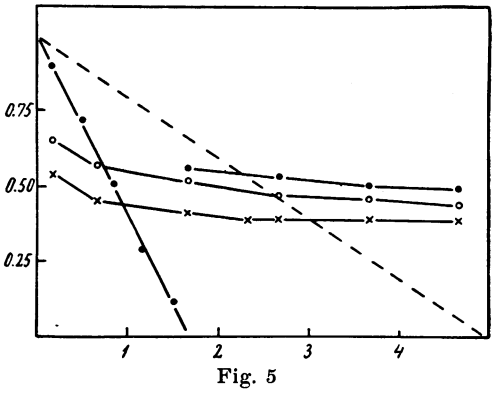

Fig. 5

Exps. on alcohol oxidation in isolated hind-limb preparations. Abscissas: time in hours. Ordinates: blood alcohol in per thousand.

The three curves from three different experiments on hind-limb preparations may be compared with a curve from an experiment on a liver (steep curve in the left part of the figure). As, however, the total amount of blood and tissue acting as solvent for the alcohol is three times as large in the hind-limb preparations as in the liver preparations, the curves from the experiments on hind-limb preparations actually must be compared with the broken curve.

That the supposition I have sketched is undoubtedly correct appears from certain experiments, as yet unpublished, which I have made on alcohol oxidation in artificially perfused isolated livers and hind-limb preparations. These experiments show that, in the liver, alcohol undergoes a rapid but primarily only partial oxidation, whereas alcohol is not affected oxidatively at all in a hind-limb preparation. I shall take the liberty of showing you one or two curves from these experiments.

From the curves (Figure 4) it will be seen that the concentration of alcohol in the blood which is perfused through a liver falls quickly and at quite a constant rate. In the course of ninety minutes the entire quantity of alcohol (300 mg.) has disappeared. The quantity of oxygen necessary to oxidize 300 mg. alcohol completely is 440 cc. The oxygen absorption in the first ninety minutes was only 225 cc. After adding the alcohol, the

respiratory quotient falls from 0.69 to 0.37. This extraordinarily low quotient can only be explained as being due to incomplete oxidation. The lack of conformity between the quantity of disappeared alcohol and the absorbed quantity of oxygen may be explained in the same manner. In addition, the alkali reserve of the blood falls as a sign that acid products are being formed. When the alcohol has almost disappeared from the blood the alkali reserve begins to increase again. At the same time the respiratory quotient also rises; in the last determination in this experiment it reaches a value of 0.9. The increase of both the alkali reserve and the respiratory quotient must be assumed to be the result of a complete oxidation of the partially oxidized acid products formed in the first period after adding the alcohol. Incidentally, the similarity between an experiment of this kind and the yeast experiments previously mentioned is very striking.

The acid product formed in the liver experiments was not isolated and identified, but there can hardly be any doubt but that it was acetic acid. As acetic acid oxidizes with great rapidity in the living organism, it may be assumed that the acetic acid formed in the liver of an intact animal after the addition of alcohol is carried with the blood to all the different tissues and oxidized there.

In an isolated hind-limb preparation, alcohol is not affected oxidatively at all. After diffusion balance is established we see only a very slow fall in the blood alcohol concentration, a fall which does not exceed that seen in control experiments, in which the blood is circulated through the apparatus without passing living tissue (Figure 5).

The absolute quantity of alcohol oxidized in an isolated liver, per gram of liver tissue and minute, averages no more than 60 per cent of the quantity oxidized in the entire animal per gram of liver tissue and minute. Probably the cause of this is that the oxygen consumption of the liver, that is to say the metabolism intensity, falls distinctly in the course of the first five or ten minutes after isolation, so that we must also assume that the alcohol oxidation in a liver in situ is greater than in an isolated liver. The dominating rôle played by the liver in alcohol oxidation also appears from the fact that in eviscerated animals, the alcohol oxidation is extremely low and does not exceed 10 per cent of that of intact animals.

Thus the assumption that the oxidative metabolism of the active muscles is of a specific nature cannot find support in the circumstance that the intensity of alcohol oxidation is not increased during muscular

exercise; for the explanation is that alcohol is only primarily affected oxidatively in the liver, so that it is the primary oxidation in the liver that becomes the limiting factor in the alcohol oxidation of the total organism.

As I have said, it may be assumed that the partially oxidized product formed in the liver can be oxidized completely in the various tissues, the muscles included. I point this out, even if at the moment I cannot submit direct experimental evidence of it, because I am inclined to think that the same applies to the oxidation of fatty acids. It is probable that the high-molecular fatty acids are not attacked, or not readily attacked oxidatively in the muscles. On the other hand, partly oxidized products of the fatty acids, such as β-hydroxybutyric acid and other ketone bodies, are very readily oxidized in the muscles. The fact that the oxidation of ketone bodies in the muscles is increased when the muscular metabolism is stimulated by work has been proved by experiments performed by Blixencrone-Moller in my institute. As these investigations have not yet been published I shall merely deal with them very broadly.

If a cat-liver with a high fat content and poor in glycogen is artificially perfused, that liver will yield very considerable quantities of ketone bodies to the blood. A liver from a cat which for some days has been treated with phloridzin, or a liver from a pancreatectomized cat, will in the course of two hours produce and transfer to the perfusion blood from 200 to 300 mg. of ketone bodies or even more. Now if such blood, which is rich in ketone bodies, is perfused through an isolated hind-limb preparation, the total quantity of ketone bodies will disappear in the course of one and one-half to two hours. This means that the quantity of ketone bodies capable of being formed by a liver, even under circumstances in which that formation is profuse, can be got rid of at a rate corresponding to the rate of formation in a mass of muscle representing less than half of the total muscle of the animal. If the experiment is performed so that the musculature of the hind-limb preparation is stimulated for a period of fifteen minutes, whereby the oxygen consumption of the preparation rises to about four times the basal consumption, all the ketone bodies will have disappeared in the course of only twenty to thirty minutes. Therefore it is not open to doubt that ketone bodies are utilized as a fuel in muscular exercise. It must be regarded as very improbable, not to say out of the question, that ketone bodies in an isolated muscle preparation are converted into carbohydrate before they are utilized.

The assumption that fat oxidation proceeds in such a manner that the fatty acids in the liver undergo a partial oxidation to β-hydroxybutyric acid, and that it is this partly oxidized product which is utilized in the muscles, provides a natural and plausible explanation of the circumstance that the efficiency in muscular exercise is lower with pure fat combustion than with chiefly carbohydrate combustion, as has been proved by Krogh and Lindhard[1].

In the course of this lecture I have referred to experimental results from very different fields within physiology: from research on the oxidative metabolism of yeast; from experiments with muscle poisoned by iodoacetic acid; the concentration of blood lactate in man during muscular exercise; the metabolism of alcohol in the organism; and the metabolism of fat in the isolated liver. To me, all these results, however different in nature they may seem, have been of significance in my consideration of the problem of the aerobic metabolism of muscle.

There is no doubt that this problem is capable of elucidation in many other and better ways. I have mainly quoted results from fields in which I myself have worked experimentally, and which therefore have played a very special part for me in my deliberations. In my opinion, all the various relations I have submitted to you are comformable, in that they contain no basis for the assumption that the aerobic metabolism of the active muscles is a pure carbohydrate metabolism; indeed, some of them are directly contradictory. It is possible that some of you share the view I have arrived at from this, that the aerobic metabolism of the active muscles is not a pure carbohydrate metabolism, whereas others do not. I scarcely imagine that I have convinced those who do not share my view, but it has been a source of great pleasure to me to have the opportunity of going so deeply into the considerations on which my opinion of the problem is founded.

REFERENCES

1. Krogh, A. and Lindhard, J. The relative value of fat and carbohydrate as sources of energy, *Biochem. J.*, 1920, *14:* 290.
2. Bierring, E. Respiratory quotient and efficiency of moderate exercise, *Arbeitsphysiol.*, 1931, *5:* 17.
3. Boje, O. Die Blutzucker während und nach körperlicher Arbeit, *Skandinav. Arch. f. Physiol.*, 1936, *74*, Suppl. *10:* 1.
4. Fletcher, W. M. and Hopkins, F. G. Lactic acid in amphibian muscle, *J. Physiol.*, 1907, *35:* 247.
5. Du Bois-Reymond, E. H. *Gesammelte Abhandlungen zur allgemeinen Muskel und Nervenphysik.* Leipzig, Veit u. Comp, 1877, v. *2*, p. 32.

6. Meyerhof, O. Das Schicksal der Milchsäure in der Erhöhlungsperiode des Muskels, *Pflügers Arch. f. Physiol.*, 1920, *182:* 284.

7. Meyerhof, O. Über den Einfluss der Sauerstoffs auf die alkoholische Gärung der Hefe, *Biochem. Ztschr.*, 1925, *162:* 43.

8. Lundsgaard, E. Weitere Untersuchungen über die Einwirkung der Halogenessigsäuren auf den Spaltungs-und Oxydationsstoffwechsel, *Biochem. Ztschr.*, 1932, *250:* 61.

9. Lundsgaard, E. Untersuchungen über Muskelkontraktionen ohne Milchsäurebildung, *Biochem. Ztschr.*, 1930, *217:* 162.

10. Lundsgaard, E. Weitere Untersuchungen über Muskelkontraktionen ohne Milchsäurebildung, *Biochem. Ztschr.*, 1930, *227:* 51.

11. Bang, O. The lactate content of the blood during and after muscular exercise in man, *Skandinav. Arch. f. Physiol.*, 1936, *74,* Suppl. *10:* 51.

12. Newman, E. V., Dill, D. B., Edwards, H. T. and Webster, F. A. Rate of lactic acid removal in exercise, *Am. J. Physiol.*, 1937, *118:* 457.

13. Nyman, E. and Palmlöv, A. On the effect of muscular exercise on the metabolism of ethyl alcohol, *Skandinav. Arch. f. Physiol.*, 1934, *68:* 271.

14. Widmark, E. M. P. Über die Einwirkung der Dinitrophenole auf die Umsetzungsgeschwindigkeit des Athylalkohols, *Biochem. Ztschr.*, 1935, *276:* 268.

15. Le Breton, E. Signification physiologique de l'oxydation de l'alcool éthylique dans l'organisme, *Ann. de physiol.*, 1936, *12:* 169; 301; 369; 805.